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Running title: Selection in mixtures of food particles

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Highlights

- In selection during chewing, different-sized particles compete for breakage sites.
- Theoretical selection models were tested using mixtures with two particle sizes.
- 1-way model: no competition from small particles because of height disadvantage.

- 2-way model: with particle piling, mutual competition between all particle sizes.
- The 2-way model applies to brittle foods, the 1-way model to certain embedded foods.

Abstract

Objectives: Two processes underlie food comminution during chewing: (1) selection, *i.e.* every particle has a chance of being placed between the teeth and being subjected to (2) breakage. Selection decreases with particle number by saturation of breakage sites, and it depends on competition between smaller and larger particles for breakage sites. Theoretical models were tested which describe competition between various sizes X. In the one-way model, small particles cannot compete with larger ones because of their smaller height. In the two-way model, small particles may compete when piled between antagonistic teeth.

Design: Five subjects participated in one-chew experiments on cubes made of Optosil®. The critical particle number ($n_c(X)$) at which saturation starts, and the number of breakage sites ($n_b(X)$) were determined by varying particle numbers (n_X) for single-sized cubes of 1.7-6.8 mm. Using $n_c(X)$ and $n_b(X)$, the models predicted relationships between number of selected particles ($n_s(X)$) and n_X in one-chew experiments using simple mixtures with only two sizes. A fixed number (mean 6 or 26) of larger cubes (X=6.8 or 3.4 mm) was mixed with various numbers (16-1024) of smaller cubes (X=4.8, 2.4 or 1.7 mm), thus varying the factors X, n_X, and possible particle piling (for X<4 mm).

Results: The one-way model was largely followed with small numbers of smaller particles and the two-way model with large numbers.

Conclusions: The two-way model applies to chewing a food which yields a loose aggregation of different-sized particles following an initial phase, whereas other circumstances may be favourable for the one-way model. As conditions of a food bolus can be approached by embedding hard Optosil particles in a soft medium, the models will, apart from dentistry, be of interest for controlling flavour release in food engineering.

Keywords: food comminution; mastication; selection; mixture; modelling

1. Introduction

One of the major functions of chewing is to prepare food for safe swallowing by mixing it with saliva and by grinding large particles into small fragments. Due to this fragmentation, the surface area of the food is increased yielding a more efficient breakdown by enzymes in the mouth and later in the gastrointestinal tract.

The food comminution in human mastication has gained a great deal of attention in many fields of research. In the field of dentistry, chewing efficiency/performance of subjects with a natural dentition has been compared with that of patients wearing dentures for examining the extent to which chewing function is restored by dental prostheses and implants (van der Bilt, 2011; Trulsson *et al.*, 2012). The relationship between dietary demands and tooth morphology is of interest from an evolutionary point of view (Lucas, 2004). Furthermore, food comminution has the interest of the food industry as it facilitates and influences the release of flavour (Hutchings *et al.*, 2012; Kim *et al.*, 2015).

As in industrial comminution processes (Epstein, 1947), the breakdown of solid food particles during chewing can be considered as the composite result of two underlying mechanisms: *i.e.* selection and breakage (Lucas & Luke, 1983; van der Glas, van der Bilt, Olthoff & Bosman, 1987). Every chewing cycle begins with selection, in which food particles have a chance to be placed between the teeth in such a way that they are at least damaged, if not broken by the subsequent breakage process. For any particle size, the selection chance can be defined as the weight of fragments with respect to the total weight of damaged and non-damaged particles from that size. During a sequence of rhythmic chewing movements of which the basic muscle activity is generated by a central pattern generator in the brainstem (Lund, Kolta, Westberg & Scott, 1998), selection of food particles will occur unconsciously rather than consciously. Variation in particle transport by the tongue and in the engagement by the teeth will ensure a highly randomized distribution of where a particle finally engages between antagonistic teeth (van der Glas, van der Bilt & Bosman, 1992).

Two categories of factors influence the selection as well as the breakage process, *i.e.* subject-related anatomical and physiological factors, and food-related factors. The selection process will depend upon factors such as the action of the tongue and the cheeks, the tooth shape, the total occlusal area of teeth, the adhesive influence of saliva (anatomical and physiological factors), and the particle number and size (food-related factors; van der Glas, van der Bilt & Bosman, 1992, Lucas, 2004). Breakage is the process by which selected particles are fractured between the teeth into fragments of variable number and size. Breakage depends upon the tooth shape, the amount and the coordination of the muscle activity (anatomical and physiological factors), the firmness of the food, and the particle size and shape (food-related factors; van der Glas, van der Bilt & Bosman, 1992; Lucas 2004).

As oral structures including the dentition are evolutionary adapted to the available food, describing and explaining relationships between the selection process and food-related factors is of primary interest (Lucas, 2004). Chewing on colour-and-form labelled particles of various size has shown that the selection chance *S(X)* increases with the particle size *X* according to a power function

(Lucas & Luke, 1983; van der Glas, van der Bilt, Olthoff & Bosman, 1987), hence: $S(X) = v.X^w$ $(0 \leq S(X) \leq 1)$. Regardless of the subject examined, the exponent *w* is always larger than one for a food which forms a loose aggregation of particles during chewing; *w* varied within a range from 1.6 to 2.6 across both studies. The variation in *w*-values is related to inter-subject differences in anatomical and physiological factors. However, values of *w* which are consistently larger than one indicate that the selection chance always increases more than proportionally with particle size. Hence, larger particles are more easily collected by the tongue and/or captured between antagonistic teeth than smaller ones in the natural chewing process.

Apart from particle size, the selection chance also depends upon the particle number as a food-related factor. The number of breakage sites which are available on the teeth, is limited because of a limited number of antagonistic teeth. Hence, the selection chance will be smaller the more the teeth become saturated with particles (van der Glas, van der Bilt & Bosman, 1992). Since the same tooth surface is used to break particles of different size, the sites at which large particles are broken overlaps greatly with the sites at which small particles are broken. Thus, small particles and large ones must compete with each other for breakage sites.

A theoretical model has been developed to describe selection of single-sized particles as a function of their number, and this model has been confirmed in one-chew experiments for a range of particle sizes of 1.2-9.6 mm (van der Glas, van der Bilt & Bosman, 1992). For any particle size, the number of selected particles increases initially approximately linearly with the number of particles offered and then gradually levels off when the available number of breakage sites becomes saturated with particles. Chewing yields mixtures of particles in the mouth with various sizes. The model that describes the selection of single-sized particles has been extended to mixtures to describe how different-sized particles mutually compete for the breakage sites (van der Glas, van der Bilt & Bosman, 1992). Two variants of the model for mixtures are: (1) the one-way interaction model in which large particles hamper the selection of smaller ones without the reverse occurring, and (2) the two-way interaction model in which a two-way competition occurs between large and smaller particles. Piling of small particles at the engagement of the food between antagonistic teeth is then essential for enabling small particles to compete with larger ones for the breakage sites.

The aim of the present study was to test the theoretical selection models for particle mixtures experimentally. In one-chew experiments, relationships were determined between number of selected particles and the number of offered particles while being part of a simple mixture which included only two sizes. The factor of possible particle piling was varied by choosing appropriate particle sizes. Observed relationships between number of selected particles and number of offered particles were compared with those as predicted by the two selection models for mixtures.

2. Materials and Methods

2.1. Theoretical background

While details about the theoretical background can be found in a previous study (van der Glas, van der Bilt & Bosman, 1992), only main features are presented here. Table 1 shows an overview of the procedure of testing the theoretical selection models for particle mixtures.

Table 1 about here

A theoretical model describes the number of particles of any single particle size X, which is selected in a single chew, $n_s(X, n_X)$, as a function of the number of particles offered of size X, n_X. An $n_s(X, n_X) - n_X$ relationship is given by:

$$n_s(X, n_X) = n_b(X).\left[1 - \left(1 - O_1(X, 1)\right)^{n_X}\right]$$ —— equation (1),

in which $O_1(X,1)$ is the affinity factor for particles of size X and $n_b(X)$ is the number of breakage sites that is available for particles of size X. The factor $\left[1 - \left(1 - O_1(X, 1)\right)^{n_X}\right]$ in equation (1) represents the fraction of the breakage sites which is occupied when n_X particles are present. $O_1(X,1)$ is the fraction of breakage sites which is, on average, occupied by the first particle that is selected by the teeth. This fraction is given by:

$$O_1(X, 1) = S_1(X, 1)/n_b(X)$$ —— equation (2),

in which $S_1(X,1)$ is the chance of the first particle of being selected to subsequent breakage. $O_1(X,1)$ is thus the selection chance of a single particle per breakage site, and is by definition considered as a measure of the particle affinity of the oral system for size X. Particle affinity is a variable which depends upon factors such as the efficiency of the tongue action for collecting and transporting particles and the extent to which antagonistic teeth are able to capture transported particles by their morphology.

The selection model for single-sized particles (equation (1)) has been successfully applied to determine $O_1(X,1)$ and $n_b(X)$ values in one-chew experiments, using various sizes and numbers of particles (van der Glas, van der Bilt & Bosman, 1992). After pseudo-chewing movements, the subjects were unexpectedly instructed to carry out a real chew on particles with a regular shape (form-labelling; cubes in the present study; for details, see below). Undamaged, hence non-selected cubes could afterwards be distinguished from broken particles. The number of selected particles, $n_s(X, n_X)$, increases approximately proportionally with the number of offered particles, n, as long as n is not excessively large. Fig. 2 (*cf.* Results) depicts examples of $n_s(X, n_X) - n_X$ relationships using equation (1) for curve-fitting of experimental data. When the teeth become saturated for large values of n_X, the increase in $n_s(X, n_X)$ with n_X levels off to the value of $n_b(X)$, the number of breakage sites available. A useful parameter which is related to the affinity factor $O_1(X,1)$, is the critical particle number, $n_c(X)$, leaving a fraction of 1/e (*i.e.* 37%) of the breakage sites unoccupied; $n_c(X)$ is given by:

$n_c(X) = -1/\ln[1 - O_1(X,1)]$ ——— equation (3)

$n_c(X)$ is used as a criterion for reaching the stage of saturation of the teeth with particles. The number of selected particles, $n_s(X, n_X)$, starts to level off for offered numbers of particles, n_X, that exceed $n_c(X)$ (Fig.2).

The selection model for a single particle size has been extended to a model for a mixture of particle sizes. Two competition mechanisms have been considered:

1. A one-way competition in which large particles hamper the selection of smaller ones without the reverse occurring, and,
2. A two-way competition between large and smaller particles.

General assumptions in both types of models for particle mixtures are:

(i) the breakage sites of smaller particles are dispersed over the post-canine teeth and are a subset of the breakage sites of larger particles;

(ii) If particles of size X_i occupy a particular fraction of their breakage sites, they occupy an equal fraction of the breakage sites of another particle size X_j.

In the one-way competition model, it has been specifically assumed that particles of descending size are successively engaged between the antagonistic teeth and that particles of smaller size which are not yet engaged are completely free to move. Because of a smaller height, small particles can then not compete with large particles for breakage sites during a chewing cycle (Fig. 1A). Large particles are engaged first during jaw closing because of their larger height and occupy then breakage sites which are not available for smaller particles anymore. Smaller particles are engaged later during jaw closing and can only mutually compete for those breakage sites which are left by the larger particles.

Fig 1 about here

In the two-way competition model, it has been specifically assumed that small particles pile between antagonistic teeth when they are locked up in a restricted space between these teeth and further between the tongue and the cheek (*cf.* Discussion). Piling of small particles will compensate the height advantage of large particles and small particles will therefore occupy part of the breakage sites of large particles when a mixture of particles of different sizes is placed between antagonistic teeth

(Fig. 1B). Reversely, large particles will occupy breakage sites of small particles by their presence between the teeth.

According to the one-way competition model, with the general case of particles of size X_i, in a mixture with p classes of larger particle sizes ($p = 0 \ldots i\text{-}1$), the number of selected particles of size X_i ($n_s(X_i, n_{X_i})$) as a function of the number of present particles of this size (n_{X_i}) follows from:

$$n_s(X_i, n_{X_i}) = [n_b(X_i). \prod_{p=0}^{i-1} U\left(X_p, n_{X_p}\right)]. [1 - \left(1 - O_1(X_i, 1)\right)^{n_{X_i}}] \quad \text{———— equation (4)}$$

where $U(X_0, n_{X_0}) = 1$ and $U\left(X_p, n_{X_p}\right) = [1 - O_1(X_p, 1)]^{n_{X_p}}$.

$n_b(X_i)$ in equation (4) is the number of breakage sites available for size X_i if larger sizes were absent, and $O_1(X,1)$ refers to affinity factors related to particle size X_i or to the other larger sizes X_p ($p = 0 \ldots. i\text{-}1$). The factor $\prod_{p=0}^{i-1} U\left(X_p, n_{X_p}\right)$ in equation (4) represents the unoccupied fraction of the breakage sites $n_b(X_i)$ which is left for the smallest particles of size X_i by the particles of the larger sizes X_p ($p=0 \ldots i\text{-}1$). The factor $[1 - (1 - O_1(X_i, 1)^{n_{X_i}}]$ represents the fraction of the breakage sites left for size X_i, which is occupied when n_{X_i} particles are present of size X_i.

Simple particle mixtures were used in the present study including only two particle sizes, *i.e.* larger particles of size X_1 of which a constant number of n_{X_1} was present and smaller particles of size X_2 of which the number n_{X_2} was varied. For a mixture with two sizes, equation (4) reduces to:

$$n_s(X_2, n_{X_2}) = [n_b(X_2). [(1 - O_1(X_1, 1)]^{n_{X_1}}]. [1 - \left(1 - O_1(X_2, 1)\right)^{n_{X_2}}] \quad \text{———— equation (5).}$$

According to the two-way competition model, with the general case of particles of size X_i, in a mixture of j classes of particle sizes ($j = 1 \ldots. k$, including class-i), the number of selected particles of size X_i ($n_s(X_i, n_{X_i})$) as a function of the number of present particles of this size (n_{X_i}) follows from:

$$n_s(X_i, n_{X_i}) = n_b(X_i). \left[n_{X_i}. \frac{\ln\left(1 - O_1(X_i, 1)\right)}{\sum_{j=1}^{k}\left(n_{X_j}. \ln\left(1 - O_1(X_j, 1)\right)\right)} \right]. [1 - \prod_{j=1}^{k} (1 - O_1(X_j\,, 1))^{n_{X_j}}]$$

equation (6), in which $n_b(X_i)$ is the number of breakage sites available for size X_i and $O_1(X,1)$ refers to affinity factors related to the particular particle size X_i or to each of the particle sizes X_j in the mixture ($j = 1 \ldots k$). The factor $[1 - \prod_{j=1}^{k} (1 - O_1(X_j\,, 1))^{n_{X_j}}]$ in equation (6) represents the total fraction of breakage sites which is occupied by all (n_{X_j}) present particles of all sizes X_j ($j=1 \ldots. k$). The factor

$\left[n_{X_i}. \frac{\ln\left(1 - O_1(X_i, 1)\right)}{\sum_{j=1}^{k}\left(n_{X_j}. \ln\left(1 - O_1(X_j, 1)\right)\right)} \right]$ represents the fraction of the total fraction of occupied breakage sites which can be attributed to the presence of n_{X_i} particles of size X_i. For a simple mixture consisting of size X_1 with a particle number n_{X_1}, and size X_2 with a particle number n_{X_2}, equation (6) reduces to:

$$n_s(X_2, n_{X_2}) = n_b(X_2).\left[n_{X_2}.\frac{\ln(1 - O_1(X_2, 1))}{(n_{X_1}.\ln(1 - O_1(X_1, 1)) + n_{X_2}.\ln(1 - O_1(X_2, 1)))}\right].$$

$$[1 - ((1 - O_1(X_1, 1))^{n_{X_1}}).((1 - O_1(X_2, 1))^{n_{X_2}})] \quad \text{—— equation (7).}$$

The models for selection in mixtures use values of affinity, $O_1(X,1)$, for each of the particle sizes X involved and the number of breakage sites, $n_b(X)$, for the particle size X of which selection is considered. Values of $O_1(X,1)$ and $n_b(X)$ can be determined for each of the sizes X when separately present in the mouth. Thus before the number of selected particles of a particular size in a mixture could be predicted by either the one-way model or the two-way model, calibration experiments with single-sized particles were needed for obtaining the required $O_1(X,1)$ and $n_b(X)$ values, using the model for single-sized particles (equation (1)). The values of $O_1(X,1)$ and $n_b(X)$ which were obtained from calibration experiments in each subject, included the subject-specific influence of anatomical and physiological factors. Hence, the influence of these factors was also accounted for in the subsequent individual predictions of number of selected particles in mixtures according to each of the two models.

The validity of each model for mixtures was tested in one-chew experiments by comparing relationships between the number of selected particles (n_s) and the number of offered particles (n) as observed for any size in particle mixtures, with n_s-n relationships predicted by the model, using data from the calibration experiments. Simple mixtures were composed, which included only two particle sizes with a constant particle number for the larger size, X_1, and a variable particle number for the smaller size, X_2. The constant particle number for the larger size, n_{X_1}, was chosen fairly large for assuring a natural manipulation of these particles by the tongue. For example, the number of large particles was at least 4 for a size of 6.8 mm. The value of n_{X_1} approximately equalled the critical particle number of this size, $n_c(X_1)$, in each subject. Although a large fraction (63%) of the breakage sites was then occupied by the larger size X_1 alone, the smaller particles would be able to compete out the larger ones according to the two-way model, by offering large numbers of smaller particles.

Apart from number of particles, another factor of interest for testing the models was the absence or presence of a possible piling of particles at the engagement of the food between antagonistic teeth (Fig. 1). Evidence for such a piling of particle sizes smaller than 4 mm, has been obtained by comparing the size of the largest particles present in the mouth at various stages of chewing, with the jaw gape at which the jaw is decelerated (van der Bilt, van der Glas, Olthoff & Bosman, 1991). Cubes were used as particles with an initially regular shape in the present study for two reasons. First, findings on particle piling originate from cubes on which chewing was started, using the same artificial test food (Optosil) as in the present study (van der Bilt, van der Glas, Olthoff & Bosman, 1991). Second, from two regular shapes that were previously used (van der Glas, van der Bilt, Olthoff &

Bosman, 1987), the difference in particle height between different sizes is twice as large for cubes than for half-cubes. Thus, the relevance of piling of small particles could be most rigorously tested by using cubes. In order to vary possible particle piling, three types of mixtures were tested:

(1) a constant number of cubes with an edge size 6.8 mm, approaching $n_c(6.8)$, was mixed with a varying number of cubes of 4.8 mm. Both sizes do not pile;

(2) a constant number of cubes of 3.4 mm, approaching $n_c(3.4)$, was mixed with a varying number of cubes of 1.7 mm. Cubes of both of these sizes (< 4 mm) pile;

(3) a constant number of cubes of 6.8 mm, approaching $n_c(6.8)$ was mixed with a varying number of cubes of 2.8 mm. Cubes of 2.8 mm pile while cubes of 6.8 mm do not.

2.2. Subjects

The study was carried out in compliance with the Helsinki Declaration, and approved by the Ethics Committee of the University of Dundee (Ref no. UREC 13098). Five subjects, 3 males and 2 females, who gave informed consent, participated in one-chew experiments. The mean age was 30.3 years (SD 3.9). The subjects had a good general health (no medication), and a sufficiently complete natural dentition (allowing missing third molars) with normal occlusal relationships. Temporomandibular disorders (jaw muscle pain and/or pain in the temporomandibular joint), or disturbances of intra-oral or peri-oral sensory function were absent.

2.3 Test food

Using brass moulds, cubes were made of Optosil® (Bayer, Germany; version 1980), a silicone dental impression material which has a constant consistency, is not affected by saliva, and of which the particles can be labelled by form and colour (van der Glas, van der Bilt, Olthoff & Bosman, 1987). Optosil (version 1980) is representative for natural brittle hard foods which form a loose aggregation of particles during chewing. Young's modulus *E*, a measure of elasticity, indicates that Optosil (E: 7.1 MPa; Olthoff, van der Bilt, de Boer & Bosman,1986; van der Glas, Al-Ibrahim & Lyons, 2012) is stiffer than raw vegetables like raw turnip ((5.8 MPa) or raw carrot (4.9 MPa), but less stiff than nuts like Brazil nuts (33.8 MPa),blanched almonds (21.6 MPa) or cashews (11.1 MPa; Agrawal, Lucas, Prinz & Bruce, 1997).

The procedure of preparing Optosil particles with a regular shape has been described in detail previously (van der Glas, Al-Ibrahim & Lyons, 2012). The ratio between Optosil base and catalyst ((Heraeus Kulzer GmbH, Hanau, Germany) was 0.02233 in the present study (*i.e.* 22.33 mg catalyst to 1 gram of base). Before mixing with catalyst, the Optosil base was mixed which three water-insoluble aluminium-bound dyes with primary colours (red, yellow and blue; in total 0.7% weight) yielding brown cubes of the same hardness as in previous studies with coloured Optosil (van der Glas, van der Bilt, Olthoff & Bosman, 1987; van der Glas, Al-Ibrahim & Lyons, 2012). Five cube sizes were prepared of which the edge size was 1.7, 2.4, 3.4, 4.8 and 6.8 mm.

2.4 One-chew experiments

Each subject was asked to attend two 1.5 hr sessions. In the first session, one-chew experiments were carried out as calibration experiments using five single cube sizes (range: 1.7-6.8 mm) to determine $O_1(X,1)$ and $n_b(X)$ values for each particle size (Table 1). In the second session, one-chew experiments were carried out using the simple cube mixtures to test the theoretical selection models for particle mixtures.

Table 2 shows the feeds (*i.e.* batches with a particular composition of cube size(s) and number(s)) and the number of trials per feed used for the calibration experiments (first session), and Table 3 those used for the simple cube mixtures (second session).

Table 2 and Table 3 about here

Each feed was placed in a separate labelled cup. Small numbers of cubes of a particular size in feeds were prepared by counting. The mean weight per cube obtained after counting of many small samples, was then used as a reference for preparing large samples of the same cube-size in the feeds by means of weighing. All cube samples were weighted with an accuracy of 1 mg. Furthermore a series of labelled containers was prepared for collecting the outcome particles after one-chews. These containers were each provided with a household sieve by which a labelled coffee filter (with a round bottom) was supported. The subjects were blinded for the labelling on the cups, containers and coffee filters.

The observer was seated in a ¾ portrait direction with respect to the subject, at a distance of 2 meters, for observing the movement of the subject's lower jaw without stressing the subject. The subject's chin was provided with a marker to facilitate observation of the jaw movement. The subject was sitting upright in a comfortable chair. An assistant who was seated alongside the subject helped the observer with handling of the cups with the feeds and the containers for collecting the chewing outcome. The assistant transferred the feeds one-by-one to a soup spoon, sprayed some water on the particles to wet the test food, and handed the filled spoon to the subject. The handed feeds were randomly varied regarding their combination of particle size(s) and number(s). In order to limit the duration of each session to 1 ½ hours, repetitions of each feed were not randomized but first completed before trials of another feed were started. The subject also received a container with a coffee filter for spitting out the outcome after a chew, which was replaced by another one after completing all trials of a feed. Hence, chewing outcomes from these trials were pooled in the same coffee filter.

The subject was instructed by the observer to transfer the cubes from the spoon to the tongue, and was then instructed to start pseudo-chewing to obtain a natural dispersion of the cubes in the mouth and to produce some saliva. Thus, like during real chewing, the lower jaw was opened and closed rhythmically with closed lips, with the test food in the mouth, but without hitting the food by the teeth. In order to approach the main features of real chewing as much as possible, each closing movement of pseudo-chewing included a horizontal component from the midline by which the antagonistic posterior teeth were vertically aligned at the working side. Furthermore, the subject carried out pseudo-chewing at a pace of natural chewing, hence automatically. During this pseudo-chewing the subject had to finish a closing movement as a real chew with final transport of the food to the teeth and subsequent breakage when the observer, who was awaiting the end of an opening phase, instructed the subject unexpectedly (by speaking out the word “chew”) to carry out this single real chew. The subject carried out the real chew smoothly by continuing the natural pace of the rhythmic jaw movements as much as possible. The observer varied the number of pseudo-chewing cycles randomly between 3 to 15 cycles before instructing “chew”. A few one-chew trials on particles were exercised at the start of each session before the actual experiments were carried out. Following the chew, the subject immediately spitted out the chewing outcome in the coffee filter.

2.5. Data collecting and processing

The chewing outcomes in the coffee filters were cleaned using a diluted solution of a detergent in warm water (60^{o}C) and by rinsing with warm clean water. The coffee filters were then folded as bags confining the particles inside, which were dried overnight in an oven at 60^{o}C.

The dried bags were weighted with an accuracy of 1 mg and emptied in a wire sieve. The emptied coffee filter was weighted and the weight of the content of the filter bag equalled the difference between the weight of the bag including content and the weight of the empty coffee filter. For particles from the calibration experiments, a single sieve was selected which only retained the cubes which were undamaged (non-selected in the one-chew trials) and large fragments of similar size. Following hand-sieving, this sieve was emptied on a sheet of smooth paper, on which damaged and non-damaged particles were separated by visual inspection, and the weight of the non-damaged particles was determined. An optimal separation of damaged and non-damaged particles was ensured by four measures: (1) following preparation in moulds, the persons who counted and weighted the cubes eliminated ones with preparation defects (layering, a non-smooth surface or edge, or one or more rounded corners), thus diminishing particle ‘noise’ and giving persons who were also particle observers following the one-chew experiments, experience on how such defects look like; (2) following one-chew experiments, all sides of each cube from the original size class were carefully inspected; for cubes of smaller size (≤ 3.4 mm) using a magnification glass, and a flat plastic (a dental instrument with blunt mini spatulas) for exposing all sides by rolling the cubes, and for gently pressing cubes for inspecting cracks more precisely; (3) inspected cubes were divided across four categories: (i) cubes undamaged by teeth and without preparation defects, (ii) occasionally cubes certainly undamaged by teeth but with a preparation defect, (iii) cubes certainly damaged by teeth, and (iv) occasionally cases of doubt which were subjected to a panel judgement from different observers; (4) particle observers were calibrated by initially re-inspecting the particles from their categories by another observer. Hence data scatter in the number of particles selected by the teeth for breakage was largely, if not entirely due to the use of a limited number of trials and/or a limited number of particles per trial in the experiments.

For particles from the experiments using mixtures, hand-sieving was first carried out using a single sieve aperture which retained non-damaged cubes of the larger size and subsequently with a sieve aperture which retained non-damaged cubes of the smaller size.

For each combination of cube size X and number n_X from each sample in a feed (one sample for calibration experiments, Table 2; two samples for mixture experiments, Table 3), the selection chance $S(X, n_X)$ was determined by:

$S(X, n_X) = (W_F(X, n_X) - W_U(X, n_X))/W_F(X, n_X)$ ——— equation (8),

in which $W_F(X, n_X)$ is the total weight of the sample with cube size X and number n_X from the feed (the total-weight of these cubes in the cups, before starting the chewing trials of the feed) and $W_U(X, n_X)$ is the weight of the pooled undamaged, hence non-selected cubes of size X after the repeated single chews on the feed. Hence, the difference between the total weight of the sample before the single chews and that of the undamaged cubes after the chews $(W_F(X, n_X) - W_U(X, n_X))$ in equation (8) was considered as the weight of damaged cubes and fragments, *i.e.* the total weight of the selected particles. As no colour labelling of different initial cube sizes was used (all cubes were brown), the size origin of the fragments was unknown in the mixture experiments with two cube sizes. Using equation (8), the selection chance was therefore calculated with respect to the total weight of the sample ($W_F(X, n_X)$ in the denominator of equation (8)), rather than to the weight of the content in the coffee filter, thus ignoring the small loss in this content with respect to the feed. In the calibration experiments, this loss was small indeed, *i.e.* on average 0.05% for the largest cubes (6.8 mm) and 0.5% for the smallest cubes (1.7 mm). For each combination of particle size and number, the number of selected particles ($n_s(X, n_X)$ was determined by:

$n_s(X, n_X) = n_X . S(X, n_X)$ ——— equation (9), in which n_X is the number of particles of size X which have been offered per trial and $S(X, n_X)$ is the selection chance of size X given by equation (8). The processing of the weight values and the automatic calculation of $n_s(X, n_X)$ values was programmed in a custom-made Excel® worksheet.

The $n_s(X, n_X) - n_X$ relationships belonging to the four n_X-values which were tested per cube size X in the calibration experiments, were curve-fitted, using non-linear regression with equation (1) (selection model for single-sized particles), in GraphPad Prism 6.07 (Graphpad Software Inc, SanDiego, CA; stage 1 in Table 1). Fig. 2 shows examples of this curve-fitting, For each subject, the curve-fitting yielded assessments of the values of $n_b(X)$ and $O_1(X,1)$ for the five cube sizes tested.

In order to restrict the duration of a session with calibration experiments to 1 ½ hours, the number of data points in the $n_s(X, n_X) - n_X$ relationships was restricted to 4 (*i.e.* 2 points in the approximately linear part of the relationship and 2 points in the part with levelling-off) rather than 6-7 points previously (van der Glas, van der Bilt & Bosman, 1992). Furthermore, the minimal number of trials for large particle numbers was restricted to 2 rather than to 6 (Table 2). Thus the assessed values of $n_b(X)$ and $O_1(X,1)$ following curve-fitting with equation (1) were less accurate than previously.

However, the relationship between the number of breakage sites, $n_b(X)$, and the particle size X can adequately be described by a power function (van der Glas, van der Bilt & Bosman, 1992), hence:

$n_b(X) = k.X^{-g}$ ——— equation (10)

Furthermore, the relationship between the critical particle number, $n_c(X)$ (related to the affinity parameter $O_1(X,1)$, equation (3)), and X can also adequately be described by a power function:

$n_c(X) = m.X^{-h}$ ——— equation (11)

Curve-fitting of $n_b(X)$-X relationships with equation (10), using the initially assessed values of $n_b(X)$ following curve-fitting of $n_s(X, n_X) - n_X$ relationships with equation (1) yielded assessments of the exponent g and the multiplication factor k, thus summarising the $n_b(X)$-X relationship for each subject (Fig. 3A). Assessments of function values of $n_b(X)$ were then obtained at the various X values from the calibration experiments by using equation (10) including a substitution of the assessed values of g and k (stage 2 in Table 1). Because of the use of information from all data points for assessing the parameters g and k of equation (10), function values of $n_b(X)$ according to this equation were more accurate than the initially assessed values of $n_b(X)$. For improving accuracy, the initially assessed values of $O_1(X,1)$ using curve-fitting with equation (1), were converted to $n_c(X)$ values using equation (3) for enabling curve-fitting of $n_c(X)$-X relationships with equation (11) and an assessment of the parameters h and m of this equation (Fig. 3B). Assessments of function values of $n_c(X)$ were then obtained at the various X values from the calibration experiments by using equation (11) including a substitution of the assessed values of h and m. The function values of $n_c(X)$ were converted back to $O_1(X,1)$-values using the relationship:

$O_1(X,1) = 1 - e^{1/n_c(X)}$ —— equation (12), which follows from equation (3).

Function values of $n_b(X)$ and $O_1(X,1)$ were then used to predict selection for the cube sizes in mixtures, starting with selection in varying numbers of smaller cubes in the presence of a constant number of larger cubes (stage 3 in Table 1). Function values of $n_b(X)$ and $O_1(X,1)$ related to X_1 and X_2, the larger and the smaller cube size in a mixture, were substituted in equation (5) of the one-way model and in equation (7) of the two-way model. The selection of cubes of the smaller size X_2 in the presence of a constant number of larger cubes of size X_1 was described as an $n_s(X_2, n_{X_2})/n_b(X_2) - n_{X_2}$ relationship, in which n_{X_2} is the varying number of offered cubes of the smaller size X_2. The number of selected cubes of the smaller size, $n_s(X_2, n_{X_2})$, was normalized by the number of their breakage sites, $n_b(X_2)$ (yielding $n_s(X_2, n_{X_2})/n_b(X_2)$), for facilitating a comparison between the three types of mixtures. The ratio $n_s(X_2, n_{X_2})/n_b(X_2)$ varies within a range from 0 to 1, regardless of size X_2. The values of $n_s(X_2, n_{X_2})/n_b(X_2)$ and n_{X_2} were further transformed to logarithmic values to obtain a variance of the $\log(n_s(X_2, n_{X_2})/n_b(X_2))$ values which was similar across the entire range of $\log(n_{X_2})$ values. The calculation of the predictions was programmed in a custom-made Excel® worksheet.

Fig. 4 (solid curves) shows examples of predicted relationships (double-log scales) between $n_s(X_2, n_{X_2})/n_b(X_2)$ and n_{X_2} for the smaller size in the three types of mixtures. It is notable that when the number of cubes of the smaller size X_2 (X_2 = 4.8, 1.7 and 2.4 mm respectively, from A to C in Fig. 4) becomes large, the end level of the normalized number of selected particles of size X_2 (n_s/n_b) is smaller for the one-way model (green solid curve) than for the two-way model (red solid curve). This feature reflects that part of the breakage sites of smaller particles are irreversibly occupied by larger particles in the one-way model and are thus not available anymore for the smaller particles. Such a restriction in availability of breakage sites for the smaller particles does not occur in the two-way model. When the smaller particles are present in large amounts, large particles will be competed out for the breakage sites, and n_s/n_b for the smaller particles approaches then even the end level of the smaller particles when present solely in the mouth (Fig. 4, blue solid curve; single-size model).

In order to determine which model describes best the empiric $\log(n_s(X_2, n_{X_2})/n_b(X_2)) - \log(n_{X_2})$ relationships from the experiments with mixtures, the Mean Square Difference (MSD) was determined between the observed $\log(n_s(X_2, n_{X_2})/n_b(X_2))$ values and the corresponding predicted values from each of the models (stage 4 in Table 1). To that end, the square difference value (D^2) was calculated between each observed value of $\log(n_s(X_2, n_{X_2})/n_b(X_2))$ and the predicted one according to the model of consideration. Hence D^2_i for a particular number of offered particles of size X_2 in a mixture ($n_{X_{2,i}}$) is given by:

$$D_i{}^2 = [\left[\log\left(\frac{n_s\left(X_2, n_{X_{2,i}}\right)}{n_b(X_2)}\right)\right]_E - \left[\log\left(\frac{n_s\left(X_2, n_{X_{2,i}}\right)}{n_b(X_2)}\right)\right]_T]^2 \quad \text{—— equation (13),}$$

in which i refers to the i^{th} data point related to cube number $n_{X_{2,i}}$, and E and T to the experimental and the theoretical value respectively of $\log(n_s(X_2, n_{X_{2,i}})/n_b(X_2))$. The Mean Square Difference (MSD) is given by:

$$MSD = (\sum_{i=1}^{k} D_i^2)/k \quad \text{—— equation (14),}$$

in which k is the number of data points across which values of $D_i{}^2$ were averaged. MSD has been determined for ranges of data points of which the values of $n_{X_{2,i}}$ are either smaller than the critical particle number of size X_2, $n_c(X_2)$ (further denoted as 'small numbers' of X_2), , or larger than $n_c(X_2)$ ('large numbers' of X_2). Being a mean, MSD values can be compared between both ranges of data points, regardless of the number of data points that has contributed to MSD. The closer the value of MSD from a model is to zero, the better is the description of the experimental data by that model.

Apart from examining how the number of selected cubes from the smaller size varies with the number of offered particles from that size in the presence of a constant number of the larger size, it has also been examined how many of these larger cubes were selected in the presence of a varying number of smaller cubes (stage 3 in Table 1). Selection of the larger size is not influenced by the presence of any smaller cubes, according to the one-way model. Hence, with a constant number, the predicted number of selected cubes of the larger size will then be constant at a level given by equation (4) of the one-way model, which is for X_0 (only a single size of 'large' cubes in a mixture) reduced to equation (1) of the single-size model (Fig. 6, green horizontal lines). The number of selected cubes of the larger size was predicted according to the two-way model, using equation (7) and the values of $O_1(X,1)$ and $n_b(X)$ from the calibration experiments for both cube sizes. In equation (7), the index '2' of variables was then related to the larger cube size and the index '1'to the smaller size. The predicted number of larger cubes according to the two-way model approaches that of the one-way model when the number of the smaller size is small (small distance between the red curves and the green lines in Fig. 6). The predicted number of selected cubes of the larger size will decrease with larger numbers of smaller cubes, according to the two-way model (Fig 6, red curves bending downwards).

In order to determine which model describes best the empiric $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationships from the experiments with mixtures (in which X_L refers to the larger size and X_S to the smaller size), the Mean Square Difference (MSD) was determined between the observed

$\log(n_S(X_L, n_{X_L})/n_b(X_L))$ values and the corresponding theoretical values from each of the models (stage 4 in Table 1).

2.6. Statistical analysis

Statistical analyses were performed using Graphpad software (Graphpad Prism 6.07; Graphpad Software Inc., San Diego, CA). For each type of mixture, MSD may depend on two factors, *i.e.* the theoretical model used ('model' factor; two levels) and the range of numbers *n* of offered particles of the smaller size ('*n*' factor; two levels: 'small' and 'large' *n*-values). The significance of the effect of each these two factors and their interaction, was determined using two-way ANOVAs with paired observations for both factors. Bonferroni's multiple comparison tests were subsequently used to determine significance of differences between inter-model or inter-*n* conditions. The level of significance was 5%.

An optimal description of experimental data points by a model is characterized by an MSD value which is close to zero. Even with an optimal description, a small residual value of a mean MSD will occur at a group level because of some scatter in the experimental data which is related to a limited number of trials per feed in individuals and to a subject sample of limited size. A residual value of the mean MSD was most closely approached in the present study by the condition with the minimum in MSD values of all conditions. In order to determine which of the other conditions had also a nearly residual MSD value, the mean MSD value of each of these conditions was compared with the one of the minimal mean MSD using a two-sample Student's t-test for paired observations. A correction of the level of significance for paired multiple comparisons with the reference condition was not applied. Hence, by using Fisher's Least Significance Difference (LSD), the incidence of conditions with a significantly different mean MSD may have been overestimated. However, the aim was to identify conditions with a pertinent lack of significance, which had, in general, a similarly small residual mean MSD as the reference condition, and also a nearly optimal match between experimental data and a model.

3. Results

3.1 Calibration experiments

Fig. 2 shows two examples of n_s-n relationships from calibration experiments with single-sized cubes. Curve-fitting of these relationships using equation (1), yielded initial estimates of the values of n_b, O_1 and n_c for various sizes. The accuracy of these initial estimates was increased by using function values which were obtained by means of curve-fitting of n_b-X and n_c-X relationships with a power function (Fig.3). Table 4 shows the parameters of the power functions describing the n_b-X and the n_c-X relationships in the various subjects, and function values of n_b and n_c at various single cube sizes. Function values of n_b, n_c and O_1 (derived from n_c) from each subject were used to predict n_s/n_b-n relationships in the mixtures, using the models.

Fig.2, Fig.3 and Table 4 about here

3.2. Experiments with simple particle mixtures

As an example of individual results, Fig. 4 depicts on double-log scales the observed $n_s(X_S, n_{X_s})/n_b(X_s) - n_{X_s}$ relationships (selection of the smaller particle size X_S) from the experiments with the mixtures of subject no. 1, and the predictions of these relationships. In the $n_s(X_{4.8}, n_{4.8})/n_b(X_{4.8}) - n_{4.8}$ relationship from the mixture of cubes of 6.8 mm and 4.8 mm (no piling of both sizes; Fig. 4A), the two-way model was followed by the data points for a larger range of numbers of the smaller size than for the other mixtures.

Fig. 4 and Fig. 5 about here

For the three types of mixtures, the two models and the two ranges of numbers of the smaller size, Fig. 5 shows the values of the Mean Square Difference (MSD), averaged across all five subjects, between the observed data and predicted data of selection of the smaller size. Fig. 5 also shows the results of statistical testing of differences between mean MSD values and the fraction of subjects with a positive inter-model difference in individual MSD values (D+, one-way model minus two-way model). A positive difference reflects a better description of the observed data by the two-way model with respect to the one-way model.

For the mixtures of 6.8 and 4.8 mm cubes (Fig. 5A), the effects of the model and the range of the offered number n of the smaller size, and interaction between both factors were significant in a two-way ANOVA. Furthermore, the mean MSD-value was smaller for the two-way model than for the one-way model, in particular for large n-values where the MSD-value of the one-way model was significantly ($p<0.01$) larger than that for small n-values, and the inter-model difference was significant ($p<0.001$). The shifts in inter-n MSD values which were in favour of the two-way model, occurred concomitantly with a shift towards an entire subject fraction with individually a positive inter-model difference in MSD (D+ from 3/5 to 5/5 subjects). The levels of mean MSD of the two-way model were residually low for the two ranges of n-values, *i.e.* they differed not significantly from the reference with the minimum level of mean MSD from all conditions in the present study. This reference (mean MSD: 0.00650, SD 0.00933) occurred for the condition: mixture 6.8 and 2.4 mm, selection of the small size, one-way model, and range of small *n*-values of the smaller size (Fig. 5C, left, black bar). Together with a significant inter-*n* change from a small mean MSD value of the one-way model to a large one (Fig. 5A), the finding of residually small mean MSD values of the two-way model for both *n*-ranges (the smallest one for the range of large *n*-values) reflected some transition from the one-way model to the two-way model in the range of small *n*-values. Beyond the range of small *n*-values, the experimental data were then well described by the two-way model for the range of large *n*-values. The smallest mean MSD value of all conditions of testing the two-way model occurred for selection of the 4.8 mm cubes in the mixture of 6.8 and 4.8 mm cubes within the range of large *n*-values of the smaller size (mean MSD: 0.00940, SD 0.00291, Fig. 5A, right, grey bar). Fig. 4A shows an example (subject no.1) of a transition in the observed selection of 4.8 mm cubes from the one-way model to the two-way model, which was nearly complete in the range of large *n*-values of the 4.8 mm cubes.

Fig. 5B depicts the mean MSD values regarding selection of the smaller size (1.7 mm) in mixtures of cubes of 3.4 and 1.7 mm (piling for both sizes). Although the effects of 'model' or '*n*', and their interaction were not significant in the two-way ANOVA, there was some change in the pattern of mean MSD values which reflected a transition from the one-way model towards the two-way model between small and large *n*-values. Thus, while the mean MSD for small *n*-values had a low residual level for the one-way model and a higher non-residual level for the two-way model, reversely the two-way model had a low residual MSD level for the large *n*-values and the one-way model a larger non-residual level. The increase with *n* of the fraction of individual positive inter-modal differences in MSD, from 1/5 to 4/5 subjects, also reflected a transition from the one-way model towards the two-way model. The levels of mean MSD for the two-way model when describing selection of 1.7 mm cubes in mixtures of 3.4 and 1.7 mm, were somewhat larger than those for 4.8 mm cubes in mixtures of 6.8 and 4.8 mm (Figs 5 A and B). This finding indicated that, in contrast to the transition for mixtures of 6.8 and 4.8 mm, the inter-model transition for mixtures of 3.4 and 1.7 mm was not largely completed within the range of small *n*-values of the smaller size (1.7 mm), but continued across the range of large *n*-values of 1.7 mm cubes. Fig. 4B depicts such a wider transition for mixtures of 3.4 and 1.7 mm from subject no.1.

Fig. 5C depicts the mean MSD values regarding selection of the smaller size (2.4 mm) in mixtures of cubes of 6.8 and 2.4 mm (piling for 2.4 mm but not for 6.8 mm). Like for mixtures of 3.4 and 1.7 mm, an inter-*n* increase occurred in the mean MSD for the one-way model and a decrease for the two way model, indicating a transition from the one-way model to the two-way model with *n*. The increase with *n* from a low residual MSD level to a high non-residual level for the one-way model was more pronounced for mixtures of 6.8 and 2.4 mm than for mixtures of 3.4 and 1.7 mm, which was reflected in a significant ($p<0.05$) interaction between the factors 'n' and 'model' in a two-way ANOVA (Fig. 5C). Furthermore, the inter-n increase in mean MSD of the one-way model was significant ($p<0.05$). The increase with n in the fraction of individual positive inter-modal differences in MSD, from 0/5 to 5/5 subjects, also reflected a transition from the one-way model towards the two-way model. The higher, non-residual level of the mean MSD from the two-way model for large n-values of 2.4 mm cubes (Fig. 5C) with respect to the low residual level for large numbers of 4.8 mm cubes (Fig. 5A) reflected a wider transition for mixtures of 6.8 and 2.4 mm than for mixtures of 6.8 and 4.8 mm. Fig. 4C depicts, like for mixtures of 3.4 and 1.7 mm (Fig. 4B) such a wider transition for mixtures of 6.8 and 2.4 mm from subject no.1.

Fig. 6 and Fig. 7 about here

In addition to the analysis of selection of smaller size in mixtures, the selection of the larger size has also been analysed. As an example of individual results, Fig. 6 depicts the observed $n_s(X_L, n_{X_L})/n_b(X_L) - n_{X_S}$ relationships from the experiments with the mixtures of subject no. 1, and the predictions of these relationships. It should be noted that the statistical power for detecting inter-model differences in MSD-values is smaller for $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationships (hence selection within the constant number of larger cubes with size X_L as a function of number of the smaller cubes, n_{X_S}) than for $\log(n_s(X_S, n_{X_S})/n_b(X_S)) - \log(n_{X_S})$ relationships (Fig. 4; hence selection of the smaller cubes with size X_S as a function of the number of these smaller cubes in the presence of a constant number of larger cubes). Statistical power is smaller for two reasons. First, in contrast to the theoretical $\log(n_s(X_S, n_{X_S})/n_b(X_S)) - \log(n_{X_S})$ relationships which differ clearly between the two models across the entire range of $\log\left(n_{X_S}\right)$ (Fig.4), the theoretical $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationships hardly differ between both models for small values of $\log\left(n_{X_S}\right)$ (Fig. 6, *cf.* Discussion). Second, the number of large cubes of 6.8 mm which was involved in the determination of their selection was relatively small for large numbers of the smaller size for which the prediction of a $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationship differs pronouncedly between both models (Fig. 6). For example, in subject no .1, only 18 cubes of 6.8 mm were involved (6 cubes per trial times 3 trials; Table 3) when the selection chance of these particles was assessed in the presence of the largest number of smaller cubes, 384 cubes of 4.8 mm in the mixture of 6.8 and 4.8 mm. Hence, the variance in the observed number of selected cubes of 6.8 mm will be relatively large at a level with the largest inter-model difference in theoretical $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationships (*cf.* Figs.6A and 6C). Because of a smaller statistical power, differences between observed and theoretical $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationships will be less frequently significant at a group level than those from $\log(n_s(X_S, n_{X_S})/n_b(X_S)) - \log(n_{X_S})$ relationships.

For the three types of mixtures, the two models and the two ranges of numbers of the smaller size, Fig. 7 shows the MSD values, averaged across all five subjects, between the experimental data and predicted data of selection of the larger size. Fig. 7 also shows the results of the statistical tests, and the fraction of subjects with a positive inter-model difference in MSD values. For all three types of mixtures, the mean MSD values were residually small for small *n*-values of the smaller size, regardless of the model involved. For the mixtures of 6.4 and 4.8 mm, and 3.4 and 1.7 mm in particular (smallest residual levels, Figs 7 A and B), this finding reflected a location of the experimental data points on the $\log(n_s(X_L, n_{X_L})/n_b(X_L)) - \log(n_{X_S})$ relationship for which both models predicted a similar degree of selection of the large cubes in the presence of small numbers of the smaller cubes (Fig. 6). Because of a similar prediction, the MSD values for the range of small *n*-values of the smaller size were, in general, inconclusive regarding the question which model was most valid.

Although significance was not reached for the factor '*n*' for mixtures of 6.8 and 4.8 mm ($p<0.1$; Fig. 7A), the levels of mean MSD were larger for large *n*-values than for small *n*-values. Whereas the mean MSD for large *n*-values was non-residually large for the two-way model, the difference with the reference minimal mean MSD was not significant for this mean MSD of the one-way model. This lack of significance of a mean MSD which had nevertheless a large value (Fig. 7A, right, black bar) was due to a large SEM. This large SEM was related to a large variation which occurred by chance in the estimation of selection chances of 6.8 mm cubes of which the numbers in the mixtures were most limited (*cf.* remarks above on statistical power). The value of mean MSD for large *n*-values was smaller for the two-way model than for the one-way model, and the fraction of subjects with a positive inter-model difference increased from 1/5 to 5/5 with *n*. These finding indicated that the selection of the 6.8 mm cubes in the presence of large amounts of smaller 4.8 mm cubes, was predominantly described by the two-way model. However, the large non-residual mean MSD value of the two-way model for large *n*-values also indicated some intermediate location of the observed values of selection with respect to the predictions from both models. As depicted in Fig. 6A for subject no.1, selection of the 6.8 mm cubes in the mixture of 6.8 and 4.8 mm occurred largely but not entirely according to the two-way model.

Regarding selection of the larger size (3.4 mm) in mixtures of 3.4 and 1.7 mm, the factor '*n*' was significant ($p<0.05$; Fig. 7B), which was reflected in an increase in mean MSD with *n*. This increase occurred in particular for the one-way model for which the mean MSD value was non-residually large for large *n*-values. In contrast, the mean MSD for large *n*-values remained non-residually small for the two-way model, and the fraction of subjects with a positive inter-model difference in MSD increased from 2/5 to 4/5. These findings at a group level indicated that selection of the 3.4 mm cubes was nearly optimally described by the two-way model. As depicted in Fig. 6B, for subject no.1, selection of the 3.4 mm particles in the mixture of 3.4 and 1.7 mm occurred nearly optimally according to the two-way model.

Regarding selection of the larger size (6.8 mm) in mixtures of 6.8 and 2.4 mm, the factor '*n*' was significant ($p<0.01$; Fig. 7C), which was reflected in an increase in mean MSD with *n*. This increase was more pronounced and significant ($p<0.05$) for the two-way model. Smaller values of mean MSD for the one-way model, regardless of the range of *n*-values, and only a small fractions of subjects (0/5 and 1/5 respectively) with a positive inter-model difference in MSD, indicated that the observed selection of the larger size, was more in accordance with the one-way model than with the two-way model. The large non-residual mean MSD values at large *n*-values for both models indicated an intermediate location of the observed values of selection with respect to the predicted values from both models. Fig. 6C (subject no. 1), depicts an inter-model transition from the one-way model towards the two-way model which was incomplete even with the maximal number of 2.4 mm particles (1024 per trial) in the mixtures of 6.8 and 2.4 mm.

Discussion

Although modern processed diets often yield a soft sticky food bolus, human post-canine teeth with their fairly blunt cusps are adapted in the hominid evolution to break brittle hard foods like roots and nuts anyhow (Lucas, 2004). The silicone rubber Optosil used as an artificial test food in the present study, is representative for natural foods such a raw Swedish turnip or carrot (*cf.* Materials and Methods, Test food), which form a loose aggregation of particles during entire chewing sequences rather than a bolus. Selection models developed for foods which form a loose aggregation of particles therefore provide relevant insight into the selection process of food comminution during natural chewing.

The starting point of modelling selection is quantifying the influence of the food-related factors, particle size and number. The influence of anatomical and physiological factors, which is reflected in the values of the model parameters can be specified by systemically varying these factors in other studies, either by comparing subsamples of subjects which differ preferably only in one of these factors or by experimentally changing a factor within subjects. Furthermore, as will be outlined below, the conditions of a food bolus can be approached by embedding hard Optosil particles in a soft medium. The influence of the medium on selection is then also reflected in the values of the model parameters. Hence, the selection models have the potential of feeding computer simulation studies with information needed to model the entire comminution process of food during chewing, including a transition from a loose aggregation of particles towards a food bolus. In the present study, the theoretical models for particle mixtures were most rigorously tested by carrying out one-chew experiments using simple mixtures which consisted of different sizes and numbers of Optosil cubes.

The models for mixtures include two types of parameters, *i.e.* affinity factors $O_1(X,1)$ for various particles sizes X (related to the critical particle numbers $n_c(X)$ of saturation) and numbers of breakage sites $n_b(X)$ on the teeth. The model for single-sized particles (equation (1)) allows a determination of both parameters in calibration one-chew experiments. The number of breakage sites, $n_b(X)$, observed in the calibration experiments, becomes smaller the larger the particle size (Table 4). Like in the previous study (van der Glas, van der Bilt & Bosman, 1992), the absolute value of the exponent g in the power function describing the n_b-X relationship was in general smaller than 2, on average this exponent was 1.78 (Table 4, top). A value of the exponent g which is larger than 2 would be expected if its value would be primarily determined by a combination of projected area (X^2) of individual particles and a degree of particle piling at the initiation of breakage which decreases with increasing particle size (van der Glas, van der Bilt & Bosman, 1992). The finding of a value of g which is smaller than 2, suggests that breakage of small Optosil particles is largely initiated on a monolayer of particles. Small particles, however, are piled during chewing in an early phase of closing the jaw. For example, when chewing was started on a batch of 320 Optosil cubes with an edge size of 2.4 mm, the jaw gape at the level of the first molars was constant across 120 cycles at the first fall in mandibular velocity, and amounted 6-7 mm (van der Bilt, van der Glas, Olthoff & Bosman, 1991). Some undamaged or only slightly damaged cubes of 2.4 mm were still present in the mouth after 120 cycles, being the particles of the largest size in the mixtures formed during the chewing process. Hence, the difference between the larger jaw gape at its deceleration and the size of the largest particles (particle height from 2.4 mm to $2.4\text{x}\sqrt{3}=4.2$ mm with a diagonal cube orientation) was bridged by particle piling. Because evidence for particle piling was lacking for a cube size of 4.8 mm or larger (van der Bilt, van der Glas, Olthoff & Bosman, 1991), possible piling may be restricted to particle sizes which are smaller than the bucco-lingual dimensions of the premolar or molar teeth. The tongue continues to rise towards the palate during the closing phase of the jaw following the delivery of particles on the occlusal area of the premolar and molar teeth (Mioche, Hiiemae & Palmer, 2002; Hiiemae & Palmer, 2003). Hence, the oral space which is left for these transported particles, is confined by the distance between the occlusal surfaces of antagonistic teeth (the more as the jaw is closing), and by the tongue and a cheek. Thus the transported particles are locked up, the more as videofluorographic recordings (Mioche, Hiiemae & Palmer, 2002) show that the tongue and cheek are inclined to push food particles back to the occlusal area which would stick out or escape otherwise. When a sufficient amount of small particles is present in the restricted space, they will pile. A stack of piled particles may be composed of two to six layers of small particle with a size of 1-3 mm (van der Bilt, van der Glas, Olthoff & Bosman, 1991). The finding of a value of the exponent g which is smaller than 2 in the n_b-X relationship (van der Glas, van der Bilt & Bosman, 1992; present study), suggests that when small Optosil particles are piled in an early phase of closing the jaw, they apparently slide along each other during further jaw closing, which includes a lateral movement. A monolayer may then have formed at the initiation of breakage. A value of the exponent g which is smaller than 2 further suggest that only specific tooth areas may be involved in the breakage of small particles, whereas every location is suitable to break large particles (van der Glas, van der Bilt & Bosman, 1992).

The affinity factor $O_1(X,1)$ increases with particle size X, which is reflected in the critical number of particles $n_c(X)$ which decreases with X in the calibration experiments (Table 4). Like in the previous study (van der Glas, van der Bilt & Bosman,1992), $n_c(X)$ decreases more than proportionally with the projected area (X^2) of the particles, *i.e.* the absolute value of the exponent h in the power function describing the n_c-X relationship is in general larger than 2. The exponent h was, on average, 2.44. The inability of the teeth to capture many small particles will contribute to a lower affinity the smaller the particle size. The difficulty the tongue encounters in collecting small particles is likely another reason for a low particle affinity. Furthermore, the subject's instruction how to manipulate the particles during pseudo-chewing in preparation to a real single chew might influence the values of particle affinity. In the previous study (van der Glas, van der Bilt & Bosman, 1992), the subject was instructed to keep the particles on the tongue until the subject transported the particles between antagonistic teeth following an unexpected instruction to finish a closing movement of the jaw as a real chew. The subject was allowed to have particles between the teeth (but not hitting them) during pseudo-chewing in the present study. The exponent h of the relationship n_c-X varied with a range of 1.99 to 2.85 in the present study, whereas h varied within a range of 2.50 to 3.43 previously (unpublished observations). The instruction regarding pseudo-chewing in the present study might cause less escape of small particles from the tongue to the oral cavity, which will be reflected in a smaller value of the exponent h.

Ranges of offered particle numbers of the smaller size occurred in which either the one-way model was followed regarding prediction of selection of the smaller particles, or the two-way model. Furthermore, a range of particle numbers occurred with an inter-model transition of the data points. These findings indicate that the calibration experiments using single-sized particles provide adequate information on affinity factors and number of breakage sites, which remain valid in models for mixtures. Whether the one-way model or the two-way model is most adequate to describe selection in mixtures from the present study depends on conditions like type of mixture with possibly particle piling or no piling, and the amount of particles of the smaller size.

In the mixtures with cubes of 6.8 and 4.8 mm which do not pile between antagonistic teeth during chewing (van der Bilt, van der Glas, Olthoff & Bosman, 1991), the selection of cubes of 4.8 mm follow the prediction of the one-way model only for the smallest number of present cubes of 4.8 mm. For larger numbers of 4.8 mm cubes, a transition occurs towards the prediction of the two-way model, which is then followed for the larger part of the range of numbers of present 4.8 mm cubes. Hence, the selection of the smaller 4.8 mm cubes follows mainly the two-way model when a sufficient number of these cubes is present to compete with a fairly large number of larger 6.8 cubes (approximately $n_c(6.8)$). Reversely, the selection of the larger 6.8 mm cubes also mainly follows the two-way model in the presence of varying numbers of 4.8 mm cubes.

The transition of the selection of 4.8 mm cubes between both models and the predominant role of the two-way model for two cube sizes which do not pile, might be related to different possible orientations of cubes when engaged between posterior antagonistic teeth. While large cubes of 6.8 will probably be engaged at two opposite sides (a flat cube orientation), smaller cubes of 4.8 mm might be engaged in one of three possible orientations, *i.e.* (1) at opposite sides (flat orientation), (2) at opposite edges (edge orientation), or (3) even at opposite corners (diagonal orientation). In a flat orientation which is most likely for small numbers of cubes of 4.8 mm because of a lack of support by neighbouring particles, cubes of 4.8 mm have some height disadvantage with respect to cubes of 6.8 mm. The larger cubes will then have a larger chance of being selected according to the one-way model. With larger numbers of cubes of 4.8 mm, edge and even diagonal orientation might occur more frequently because the orientation of tilted cubes can then be stabilized by the support of neighbouring particles. The height of cubes of 4.8 mm with an edge orientation is 6.8 mm (4.8 x $\sqrt{2}$), thus equal to the height of 6.8 mm cubes. The effective height with a diagonal orientation (which is however less stable and therefore will occur less likely than that with an edge orientation), is even 8.3 mm (4.8 x $\sqrt{3}$). Thus in the absence of piling and with the smallest inter-cube height disadvantage in the present study (height ratio of 1.41 between 6.8 mm and 4.8 mm cubes), cubes of 4.8 mm can overcome a disadvantage in height by a tilted orientation.

In the mixtures of 3.4 and1.7 mm and 6.8 and 2.4 mm cubes, cubes of the smaller size have a height disadvantage that is related to a height ratio of 2.00 and 2.83 respectively between both sizes in the mixture. Such height disadvantages cannot be compensated by variation in cube orientation. In the presence of a constant, fairly large number of larger cubes, the selection of cubes of the smaller size follows mainly the one-way model when the particle number of the smaller size is small. With increasing numbers of the smaller size, the selection of this size shows an inter-model transition and ultimately follows the two-way model. Reversely, the selection of the larger cubes in the mixtures of 3.4 and1.7 mm followed the two-way model within a large range of numbers of the small cubes. For the selection of 6.8 mm cubes in mixtures of 6.8 and 2.4 mm, a transition occurred from the one-way model towards the two-way model, which, however, was not complete yet with the maximal number of 2.4 mm cubes offered in the present study (i.e. 1024 particles). More 2.4 mm cubes may be needed to bridge by particle piling the largest height ratio in the present study, *i.e.* a ratio of 2.83 between 6.8 and 2.4 mm. Hence, all findings from mixtures in which piling was possibly involved for at least one size, suggest that a sufficiently large number of offered small particles will yield sufficient piling of the collected particles between the teeth for enabling competition with the larger particles for the breakage sites. As outlined above, evidence for such a piling during natural chewing has been obtained by comparing the size of the largest particles present in the mouth, with the jaw gape at which the jaw is decelerated by engaging the food (van der Bilt, van der Glas, Olthoff & Bosman, 1991).

The findings regarding the role of both models in one-chew experiments with simple mixtures, are representative for conditions of selection during a natural chewing process. Chewing is usually started on a batch of single-sized large particles, for example, eight Optosil cubes of 8 mm (Olthoff, van der Bilt, Bosman & Kleizen, 1984). The total volume of 4.1 cm^3 of these cubes yields a usual filling of a soup spoon and an optimal mouthful (Lucas and Luke, 1984). However, the present findings and the previous ones (van der Glas, van der Bilt & Bosman, 1992) from one-chew experiments with single-sized cubes show that the breakage sites on the teeth will initially be saturated with eight large-sized cubes of 8 mm. It takes therefore some chewing cycles (at least 10 cycles; Olthoff, van der Bilt, Bosman & Kleizen, 1984; van der Bilt, van der Glas, Olthoff & Bosman, 1991) before the last 8 mm cube has been selected and fragmented. The number of smaller fragments will be small during a few initial cycles so that these fragments are only a little or non-piled. In the presence of larger particles including some initial large cubes, selection of the various sizes will then occur according to the one-way interaction model.

After the initial phase of chewing, large particles will become scarce and smaller fragments of various sizes will be produced in such large numbers that particle piling will occur extensively. Selection of the various sizes will then follow the two-way interaction model. Apart from findings of one-chew experiments with simple mixtures, the validity of the two-way model in a later phase of natural chewing also follows from the model's prediction that the ratios of selection chances between two arbitrary sizes will be constant during chewing. In the natural chewing process, the selection chance $S(X)$ (ratio between the selected number and the total number of particles of size X) increases with the particle size X according to a power function (Lucas & Luke, 1983; van der Glas, van der Bilt, Olthoff & Bosman, 1987), hence: $S(X) = v.X^w$ $(0 \leq S(X) \leq 1)$. The exponent w which determines the ratios in selection chance between different particle sizes, is constant for each subject, regardless of the progress of the chewing process (van der Glas, van der Bilt, Olthoff & Bosman, 1987). Hence, the ratios in selection chance between two arbitrary sizes are constant during chewing in a later phase, a finding which is in agreement with the prediction of the two-way model but not with that of the one-way model (van der Glas, van der Bilt & Bosman, 1992).

Although particle conditions may be in favour of the one-way model during the first initial cycles when chewing is started on a batch of large particles of a food which forms a loose aggregation of particles, the difference with the two-way model regarding selection of large particles will be small because of a small affinity for small particles. Hence, small fragments will not (one-way model) or hardly (two-way model) be able to compete with the large particle for breakage sites when they are initially present in small numbers. A small difference between both models occurred indeed in the one-chew experiments when considering the number of selected particles of the larger size in the presence of small numbers of the smaller size (Fig. 6). Because the selection and the subsequent breakage of the larger particles determine mainly the size distributions by weight during the initial phase of chewing, a sufficiently accurate description of the selection of the larger particles is then most relevant for modelling food comminution. The two-way interaction model will therefore be sufficiently adequate to describe particle selection during an entire chewing sequence if chewing is started on a number of large particles of a food which forms a loose aggregation of particles.

Although Optosil has been used as an artificial test food which forms a loose aggregation of particles to date, conditions in a food bolus can be approached by embedding hard Optosil particles in a soft medium. The influence of the soft medium on selection parameters of the harder particles can be determined in one-chew experiments, after recovering of the hard particles and fragments from the medium. Embedding will influence the process of concentrating Optosil particles between teeth, *i.e.* by means of collection and transport by the tongue and locking up in a limited space between antagonistic teeth, the tongue and a cheek. Embedding in a viscous fluid or semi-solid medium (thus approaching conditions of a natural food bolus most closely) may facilitate concentrating of particles. On the other hand, embedding in a bar made of a soft solid medium may hamper concentrating, when the width of the bar is larger than the bucco-lingual dimensions of the premolar and/or molar teeth. Such a bar placed between antagonistic teeth sticks out, and the particle concentration between the teeth may therefore be lower than that following collecting from loose particles by the tongue. In calibration experiments, the influence of different types of embedding will be reflected in the model parameters of relationships between particle affinity and size and between number of breakage sites and particle size respectively. The influence of embedding will then be accounted for in model predictions for embedded particle mixtures. Apart from measuring selection parameters, the influence of a soft medium on breakage of the particles can also be determined, using form-and-colour labelling of different sizes of Optosil particles (van der Glas, van der Bilt, Olthoff & Bosman, 1987).

As outlined below, the experimental validation of the theoretical selection models for particle mixtures here provides the essential knowledge to determine the conditions for a controlled flavour release by varying particle shape, size and number ratio in mixtures and the way of particle embedding. Many foods contain a mixture of particles (and more than one ingredient food component) in a matrix. By measuring selection and breakage of particles made of an artificial test food like Optosil, under various conditions, further insight will be gained in how flavour can be released during chewing of particles of a real food, and in designing new food products.

Kim *et al.* (2015) studied the oral processing of mixtures of food particles during chewing on gelatine-based gel particles. It was found that larger particles were preferentially selected during initial phase of chewing so that the smaller particles were chewed significantly less and swallowed bigger than when chewed on their own. From this observation, a concept of a differential incorporation of ingredients in large and small particles has been outlined (Kim *et al.*, 2015). For example, it may be possible to minimize the unpleasant flavour of phytonutrients by incorporating them into smaller particles in a mixture, and to maximize sweet/salty tastes by adding sugar/salt to large particles.

The study of Kim *et al.* (2015) was not designed to distinguish between the two selection models, *i.e.* particle number was not varied in the experiments using single-sizes or mixtures. Furthermore, particles of different size were cylinders which were differently shaped, i.e. because of a constant height of 5 mm, the largest particles had a tablet-shape while smaller particles became more elongated cylinders the smaller their size was. The present study provides nevertheless clues about a possible involvement of the two-way selection model and about conditions which will increase selection according to the one-way model. In a mixture with the largest difference in particle size, 2 grams of large cylinders (diameter 15 mm, height 5 mm) were mixed with 2 grams of small cylinders (diameter 4 mm, height 5 mm; Kim *et al.*, 2015). With a density of 1.158 g/cm^3, the particle number was therefore 2 for the larger size and 27 for the smaller size. The dimensions of the smaller cylinders approach closely those of 4.8 mm cubes used in the present study. Hence, the critical particle number at which saturation of the posterior teeth starts, n_c, will be similar for the smaller cylinders and cubes of 4.8 mm. Because n_c is on average 14.5 for cubes of 4.8 mm (Table 4, bottom), 27 cylinders of the smaller size can be considered as a 'large' number (27 > 14.5) of the smaller size in the simple cylinder mixture. The present study (Fig. 4) shows that with such a large particle number of the smaller size, nearly twice the estimated value of n_c, the smaller particles can compete with the large ones for the breakage sites to such an extent that selection may follow largely the two-way model when chewing is started on a mixture of cylinders.

The conclusion of a possible involvement of the two-way model, even in the initial phase of chewing, is reinforced by considering the mutual effective heights of the two most extreme cylinder sizes used. The smallest dimension of both sizes of cylinders was so similar (4-5 mm) that particle piling was not required to bridge any difference in height between both sizes (particle piling is even unlikely with a height of 4-5 mm; van der Bilt, van der Glas, Olthoff & Bosman, 1991). The large cylinders (tablets with a height of 5 mm) will most likely be engaged between antagonistic posterior teeth in an upright orientation, *i.e.* their circular base and top with a diameter of 15 mm being engaged. Engagement of the smaller cylinders occurs possibly in (1) an upright orientation, and (2) an orientation perpendicular to the long axis (5 mm), which is larger than the cylinder diameter (4 mm). While the second more stable orientation is more likely with a small number of the smaller size, the frequency of an upright orientation will be increased by the support of neighbouring smaller cylinders when these are present in large numbers. Because the cylinders of both sizes had the same height (5 mm), any height advantage of the larger size will be lacking when both sizes are engaged in upright orientation, which favours the two-way selection model. The incidence of an upright orientation at engagement will most likely occur for cylinders of the smaller size in other mixtures for which the diameter was chosen larger than 4 mm while the height was constant at 5 mm. The two-way selection model may therefore dominate selection in all types of cylinder mixtures and all phases of chewing.

Anyhow, the observation of a smaller degree of selection of the smaller particles in a mixture than when chewed on their own (Kim *et al.*, 2015), can be explained by the two-way model in terms of a smaller degree of affinity of the oral system for smaller particles. In order to reinforce the concept of a differential incorporation of flavour ingredients in large and small particles, selection of the smaller size could further be decreased by creating food conditions which are exclusively favourable for the one-way model. Less breakage sites are then left available for the smaller size. Even in a simple mixture of loose particles, the one-way model will be favoured by choosing different sizes with an enhanced difference in particle height, for example, by using cubes for the larger size and half-cubes for the smaller size. Another way of favouring the one-way model can be achieved by certain ways of embedding of harder food particles in a soft solid medium, even in the presence of large amounts of small particles. For example, when large cubes are randomly placed in a mono-layer, the distribution of their locations in a soft solid medium will not be influenced by the presence of small cubes if these are added subsequently around previously placed large ones. When the medium is engaged between antagonistic teeth, the pre-determined and fixated distribution of the large particles will then determine the fraction of the breakage sites which will be occupied by large particles and the fraction which is left for small particles. Such a selection according to the one-way model will delay the selection and subsequent breakage of the small particles during some subsequent chewing cycles. Future studies, using the models as a theoretical frame, are necessary to elucidate the potential of using different sizes with a different particle shape and embedding of particles for controlling flavour release.

5. Conclusions

Calibration experiments using particles of single sizes provide adequate information on affinity factors and numbers of breakage sites available on the teeth for an accurate description of selection according to either the one-way or the two-way interaction model for particle mixtures. One-chew experiments using simple particle mixtures consisting of a constant number of larger cubes and varying numbers of smaller cubes show that with increasing particle numbers of the smaller size, a transition occurs from a description of selection according to the one-way model to a description by the two-way model. This finding suggest that the height advantage of the larger particles in the mixture for being engaged between the teeth is compensated for either by a tilted orientation or by piling of the smaller particles, when smaller particles become abundant. At the one hand, the conditions during a later phase of natural chewing on a food which forms a loose aggregation of particles, *i.e.* scarcely present large particles and abundantly present small particles, are favourable for the two-way model of selection. On the other hand, the conditions of particle sizes and numbers during the initial phase of chewing on such a food, are favourable for describing selection by the one-way model. Furthermore, it can be expected that particular conditions of embedded particles in a soft solid medium will also be favourable for describing selection by the one-way model. Such conditions might be of interest for controlling flavour release.

Conflict of interest

The authors report no conflict of interest.

Ethical approval

This study was approved by the Ethics Committee of the University of Dundee (ref no. UREC 13098).

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References

Agrawal, K.R., Lucas, P.W., Prinz, J.F. & Bruce, I.C. (1997). Mechanical properties of foods responsible for resisting food breakdown in the human mouth. *Archives of Oral Biology*, *42*, 1–9.

Epstein, B. (1947). The mathematical description of certain breakage functions leading to the logarithmico-normal distribution. Journal of the Franklin Institute, 244, 471-477.

Hiiemae, K.M. & Palmer, J.B. (2003). Tongue movements in feeding and speech. Critical Reviews in Oral Biology and Medicine, 14, 413-429.

Hutchings, S.C., Foster, K.D., Bronlund, J.E., Lentle, R.G., Jones, J.R. & Morgenstern, M.P. (2012). Particle breakdown dynamics of heterogeneous foods during mastication: peanuts embedded inside different food matrices. *Journal of Food Engineering, 109,* 736-744.

Kim, E.H.-J., Jacobsen, V.B., Wilson, A.J., Waters, I.R., Motoi, L., Hedderley, D.I. & Morgenstern, M.P. (2015). Oral processing of mixtures of food particles. *Journal of Texture Studies, 46,* 487-498.

Lucas, P.W. & Luke, D.A.(1983). Methods for analyzing the breakdown of food in human mastication. *Archives of Oral Biology, 28,* 813-819.

Lucas P.W. & Luke D.A. (1984). Optimum mouthful for food comminution in human mastication. *Archives of Oral Biology, 29,* 205-210.

Lucas, P. W. (2004). *Dental functional morphology,how teeth work,* 1st ed. Cambridge: Cambridge University Press.

Lund, J.P., Kolta, A., Westberg, K.G. & Scott, G. (1998). Brainstem mechanisms underlying feeding behaviors. *Current Opinions of Neurobiology, 8,* 718-724.

Mioche, L., Hiiemae, K.M. & Palmer, J.B. (2002). A postero-anterior videofluorographic study of the intra-oral management of food in man. *Archives of Oral Biology, 47,* 267- 280.

Olthoff, L.W., van der Bilt, A., Bosman, F. & Kleizen, H.H. (1984). Distribution of particle sizes in food comminution by human mastication. *Archives of Oral Biology, 29,* 899-901.

Olthoff, L.W., van der Bilt, A., de Boer, A. & Bosman, F. (1986). Comparison of force-deformation characteristics of artificial and several natural foods for chewing experiments. *Journal of Texture Studies, 17,* 275-289.

Trulsson, M., van der Bilt, A., Carlsson, G.E., Gotfredsen, K., Larsson, P., Müller, F., Sessle, B.J. & Svensson, P. (2012). From brain to bridge: masticatory function and dental implants. *Journal of Oral Rehabilitation, 39,* 858-877.

van der Bilt, A., van der Glas, H.W., Olthoff, L.W. & Bosman, F. (1991). The effect of particle size reduction on the jaw gape in human mastication. *Journal of Dental Research, 70,* 931-937.

van der Bilt, A. (2011) Assessment of mastication with implications for oral rehabilitation: a review. *Journal of Oral Rehabilitation, 38,* 754-780.

van der Glas, H.W., van der Bilt, A., Olthoff, L.W. & Bosman, F. (1987). Measurement of selection changes and breakage functions during chewing in man. *Journal of Dental Research, 66*, 1547-1550.

van der Glas, H.W., van der Bilt, A. & Bosman, F. (1992). A selection model to estimate the interaction between food particles and the post-canine teeth in human mastication. *Journal of Theoretical Biology, 155,* 103-120.

van der Glas, H.W., Al-Ibrahim, A. & Lyons, M.F. (2012). A stable artificial test food suitable for labeling to quantify selection and breakage in subjects with impaired chewing ability. *Journal of Texture Studies, 43,* 287-298.

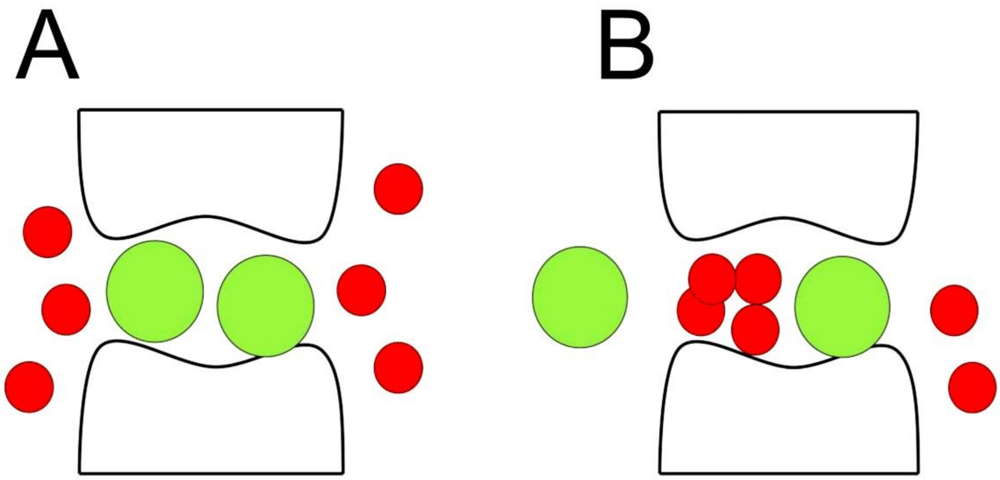

Fig. 1. Models of selection for breakage of food particles in a mixture of particle sizes, during a chew. A, one-way competition between particle sizes. Because of a larger height, large particles are engaged first between antagonistic teeth during jaw closing and occupy breakage sites while excluding small particles. B, two-way competition between particle sizes. Piling of small particles will compensate the height advantage of large particles. Small particles therefore occupy part of the breakage sites of large particles.

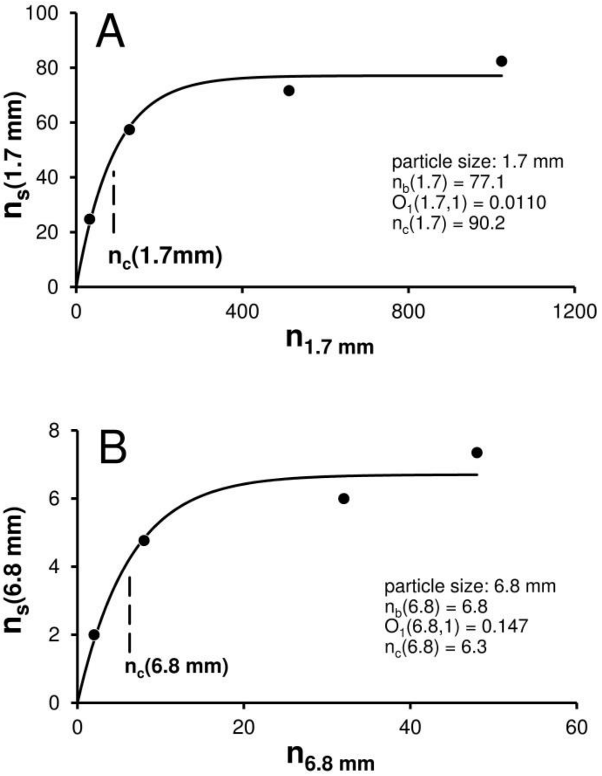

Fig. 2. Examples (subject no.1) of relationships between the number of selected particles (n_s) and the number of offered particles per trial (n), for the smallest (1.7 mm, A) and largest (6.8 mm, B) cubes tested in calibration one-chew experiments. The experimental data (dots) were curve-fitted using the selection model for single-sized particles (text, equation (1); solid curves), yielding initial assessments of the number of breakage sites, $n_b(X)$, and the affinity factor, *O1(X,1)* for the various particle sizes X. Vertical hatched line, the critical particle number, $n_c(X)$, at which saturation of the breakage sites starts. $n_c(X)$ is related *to* $O_1(X,1)$ (equation (3), text).

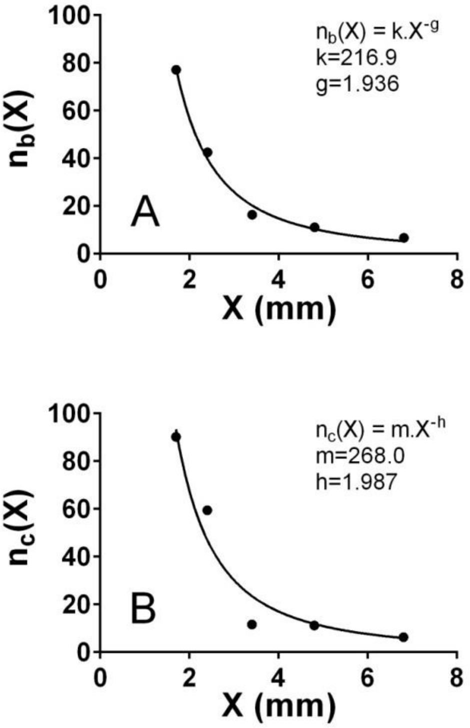

Fig. 3. Example (subject no.1) of relationships between number of breakage sites, $n_b(X)$ and particle size, X (A), and between critical particle number at which the breakage sites on the teeth becomes saturated, $n_c(X)$ and X (B). The experimental data (dots) which were initial assessments of $n_b(X)$ and $n_c(X)$ values (*cf*. Fig. 2), were curve-fitted using a power function (solid curves), yielding assessments of the function parameters and $n_b(X)$ and $n_c(X)$ values according to the functions with improved accuracy with respect to the initial assessments of $n_b(X)$ and $n_c(X)$.

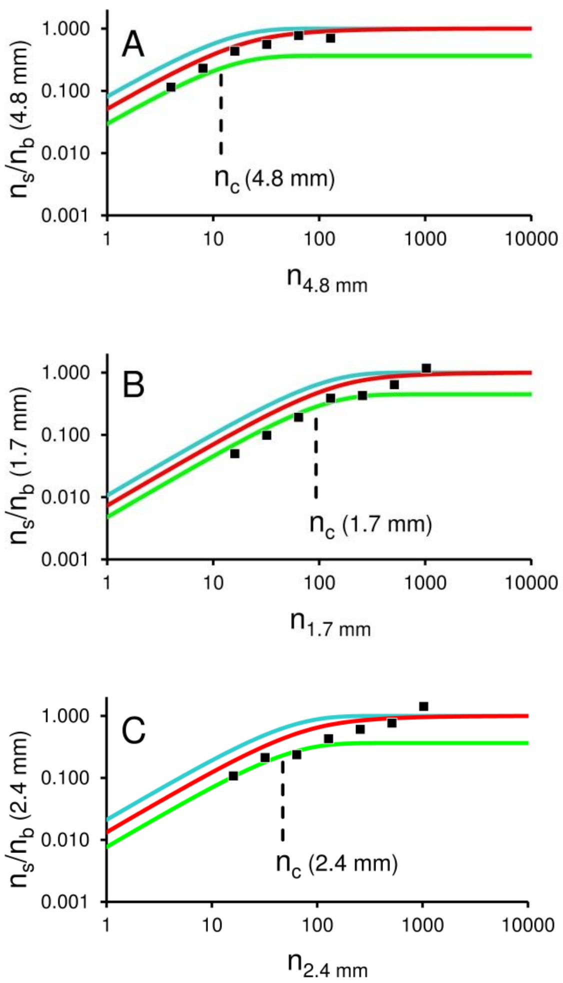

Fig.4. Example (subject no.1) of the relationships between the normalized number of selected cubes, $n_S(X_S, n_{X_S})/n_b(X_S)$ and the number of offered cubes, n_{X_S}, both variables for the smaller cube size *(X_S)*, in mixtures in which the number of the larger cubes was constant. Squares, experimental data. Vertical dashed line, *$n_c(X_S)$*, critical number of the smaller cubes. A, mixtures of cubes of 6.8 and 4.8 mm, in which the number of 6.8 mm ($n_{6.8\ mm}$) was constant at 6. B, mixtures of 3.4 and 1.7 mm, in which $n_{3.4\ mm}$ was constant at 19. C, mixtures of 6.8 and 2.4 mm, in which $n_{6.8\ mm}$ was constant at 6. Green curve, prediction of the $n_S(X_S, n_{X_S})/n_b(X_S) - n_{X_S}$ relationship according to the one-way interaction model (text, equation (5)). Red curve, prediction according to the two-way interaction model (text, equation (7)). Blue curve, prediction according to the single-size model (text, equation (1)) if larger particles were absent.

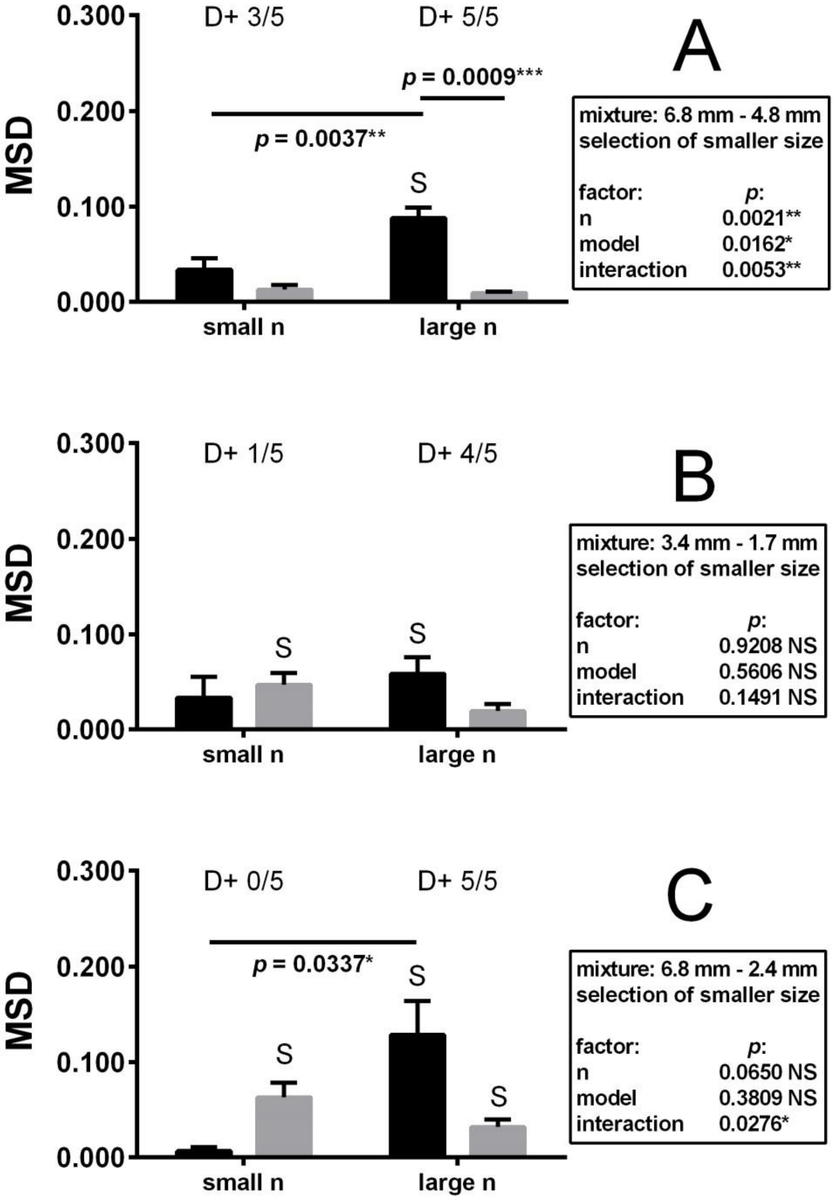

Fig.5. Mean Square Difference (MSD, mean and SEM; n=5 subjects) for the 1-way interaction model (black bars) and the 2-way interaction model (grey bars), between experimental data and predicted ones on the number of selected cubes of the smaller size in simple mixtures. Mean MSDs are depicted for the three types of mixtures and two ranges of offered numbers *n* of the smaller cube size, *i.e*. *n*-values smaller than the critical particle number for the smaller size (‘small n’), and larger *n*-values (‘large n’). The more the mean MSD approaches the value of zero, the better is the description of the experimental data by a corresponding model at a group level. S, mean MSDs which are significantly larger in a two-sample Student’s t-test (paired observations) than the minimal mean MSD observed in the present study (mean: 0.00659, SEM 0.00417, C left, black bar). Text boxes: *p*-values and significance levels from two-way ANOVAs with paired observations for the two factors per mixture: ‘n’ (two levels) and ‘model’ (two levels), and their interaction. Significance level: NS non-significance; * (p<0.05), ** (p<0.01). Horizontal bars, cases of significant differences (adjusted *p*-values with significance level) between either inter-*n* or inter-model conditions, in Bonferroni’s multiple comparison tests. D+, the fraction of subjects in which a positive difference occurred between individual MSDs from both models (one-way minus two way). A positive sign indicates a better description of the experimental data by the 2-way model.

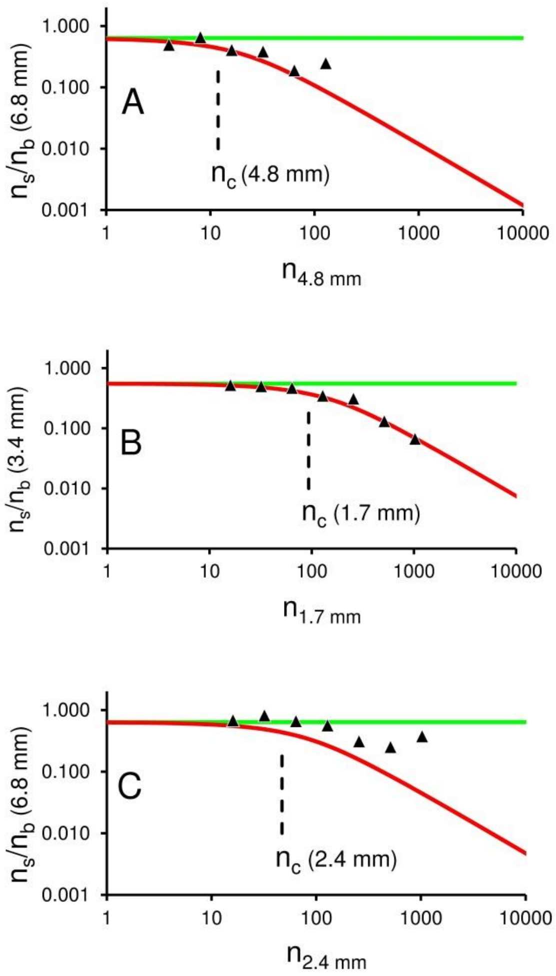

Fig. 6. Example (subject no.1) of the relationships between the normalized number of selected cubes, $n_S(X_L, n_{X_L})/n_b(X_L)$, of the larger size *($X_L$)* and the number of offered cubes, n_{X_S} of the smaller size *(X_S)*, in mixtures in which the number of the larger cubes was constant. Triangles, experimental data. Vertical dashed line, $n_c(X_S)$, critical number of the smaller cubes. A, mixtures of cubes of 6.8 and 4.8 mm, in which the number of 6.8 mm ($n_{6.8\ mm}$) was constant at 6. B, mixtures of 3.4 and 1.7 mm, in which $n_{3.4\ mm}$ was constant at 19. C, mixtures of 6.8 and 2.4 mm, in which $n_{6.8\ mm}$ was constant at 6. Green curve, prediction of the $n_S(X_L, n_{X_L})/n_b(X_L) - n_{X_S}$ relationship according to the one-way interaction model, which, for the larger particles, is identical to the single-size model (text, equation (1)). Red curve, prediction according to the two-way interaction model (text, equation (7)).

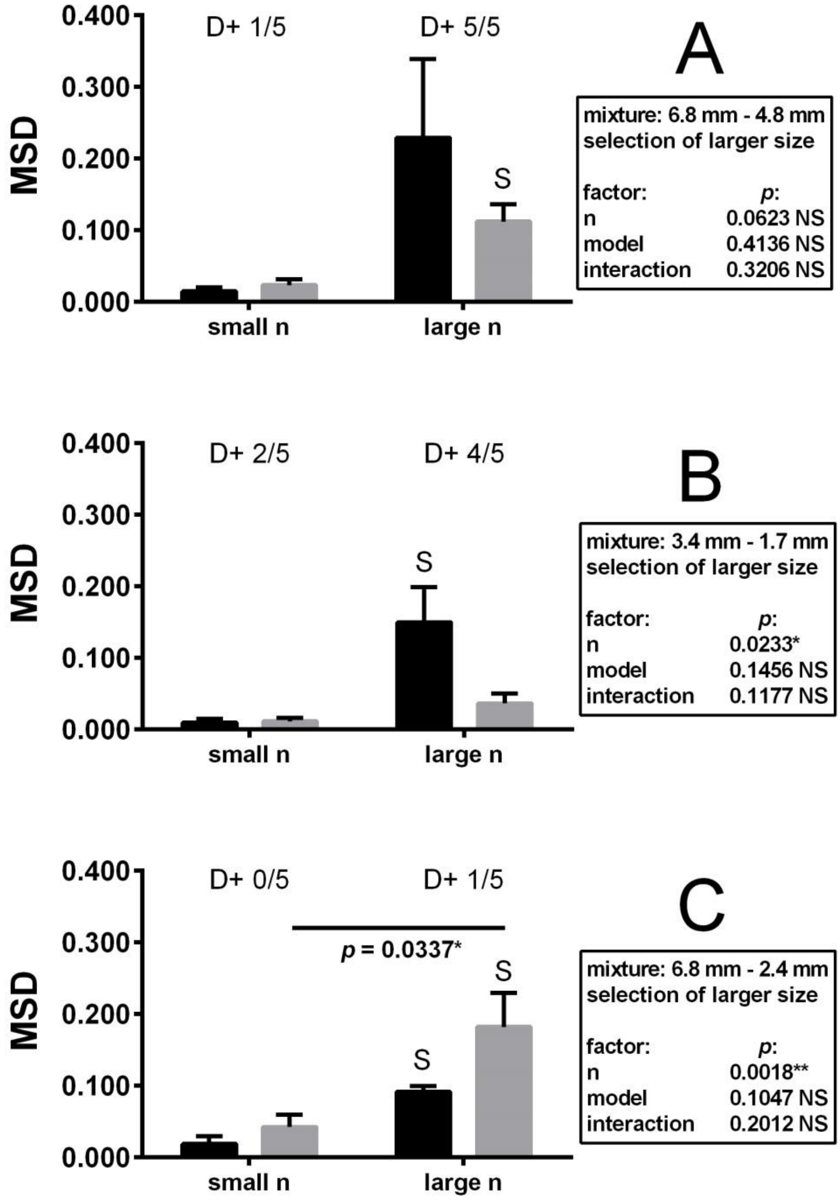

Fig.7. Mean Square Difference (MSD, mean and SEM; n=5 subjects) for the 1-way interaction model (black bars) and the 2-way interaction model (grey bars), between experimental data and predicted ones on the number of selected cubes of the larger size in simple mixtures. The MSDs are depicted for two ranges of numbers of cubes of the smaller size (small *n* and large *n*). For further explanation, see Fig. 5.

Table 1. Procedure of testing theoretical selection models for particle mixtures

Stage (1): Obtaining values of particle affinity and number of breakage sites for each subject: One-chew calibration experiments using 5 cube sizes X (1.7, 2.4, 3.4, 4.8 and 6.8 mm) and various numbers of cubes per size (*cf.* section 2.4. 'One-chew Experiments' *f.*); For each size X, determination of the relationship between number of selected particles and number of offered particles, with particle affinity, $O_1(X,1)$ and number of breakage sites, $n_b(X)$ as parameters in curve-fitting using the single-size selection model (equation (1));
Stage (2): Enhancing the accuracy of values for $O_1(X,1)$ and $n_b(X)$: Conversion of $O_1(X,1)$ values obtained in stage (1) to values of critical number of particles, $n_c(X)$ using equation (3); Curve-fitting of the $n_c(X)$ values using a power function describing the $n_c(X)$-X relationship (equation 11) and determination of the function values of $n_c(X)$ for the five cube sizes used (range: 1.7-6.8 mm); Re-conversion of the function values of $n_c(X)$ to function values of $O_1(X,1)$ using equation (12). Because the function values include information from five $n_c(X)$-values used in the curve-fitting with the power function, the accuracy of a function value of $O_1(X,1)$ for a particular particle size is larger than that of a single estimated value of $O_1(X,1)$ obtained in stage (1); Curve-fitting of the $n_b(X)$ values using a power function describing the n_b-X relationship (equation 10) and determination of the function values of $n_b(X)$ for the cube sizes used;
Stage (3): Predicting theoretical values of number of selected cubes in one-chew experiments with simple mixtures: For each subject, substitution of the function values of particle affinity, $O_1(X,1)$, and number of breakage sites, $n_b(X)$, for the cube sizes used in simple mixtures (three types of mixtures, each with two cube sizes), in equation (5), 1-way interaction model for selection, and in equation (7), 2-way interaction model respectively; For each model, calculation of predictions of the number of selected particles of the smaller size in a mixture (in the presence of a constant number of the larger size), as a function of the number of offered particles of the smaller size, using equation (5) and (7) respectively with substituted parameter values; Also calculation of the number of selected particles within a constant number of the larger size as a function of the number of offered particles of the smaller size;
Stage (4): Testing the validity of the models of selection of particles during a chew: One-chew experiments using simple particle mixtures; Determination of the Mean Square Difference (MSD; equation (14)) between log-transformed experimental values and predicted theoretical ones of the number of selected particles. Using MSD, the validity of the theoretical models is tested statistically.

Table 2. Feeds of cubes used in the calibration experiments

Cube edge size X (mm)	Number of cubes (n_X)	Number of trials
1.7	32	12
	128	4
	512	3
	1024	3
2.4	16	8
	64	4
	256	3
	512	3
3.4	8	14
	32	4
	128	3
	256	3
4.8	4	20
	16	8
	64	3
	128	3
6.8	2	25
	8	13
	32	5
	48	2

A feed included a particular cube size and cube number; its application was repeated (number of trials) to obtain a reliable estimate of the average number of particle selected. Note that a factor 4 was applied between the first three particle numbers. A factor 2 was applied between the 3^{rd} and the 4^{th} particle number, except for a particle size of 6.8 mm to restrict the maximal mouth filling to 15.1 cm^3.

Table 3. Feeds used in the experiments with simple mixtures of cubes

Edge size of larger cubes* (mm)	Edge size of smaller cubes (mm)	Number of smaller cubes	Number of trials
6.8	4.8	4	15
		8	10
		16	6
		32	3
		64	3
		128	3
6.8	2.4	16	8
		32	6
		64	4
		128	3
		256	3
		512	2
		1024	2
3.4	1.7	16	12
		32	6
		64	4
		128	3
		256	3
		512	3

A feed included two sizes and numbers of cubes; its application was repeated (number of trials) to obtain a reliable estimate of the average number of particle selected. Note that a factor 2 was applied between successive particle numbers of the smaller cube size. *The number of larger cubes was constant at approximately the critical particle number, n_c, for the larger size, which was determined in the calibration experiments. For cubes of 6.8 mm, the constant number was 6, 6, 5, 8, and 4 for subject's no. 1-4, and 6 respectively. Subject no. 5 participated in another study, together with the subjects from the present study. For cubes of 3.4 mm the constant number was 19, 24, 22, 30 and 32 respectively.

Table 4. Variables related to size-dependent particle affinity and number of breakage sites

Table 4. Variables related to size-dependent particle affinity and number of breakage sites

	$n_b(X) = k.X^{-g}$		$n_c(X) = m.X^{-h}$	
Subject no.	*k*	*g*	*m*	*h*
01	216.9	1.94	268.0	1.99
02	436.3	2.30	1171.0	2.85
03	145.6	1.71	355.7	2.02
04	93.6	1.21	1414.0	2.79
06	142.2	1.72	820.0	2.56

	Cube edge size (X, mm)									
	6.8		4.8		3.4		2.4		1.7	
Subject no.	n_b	n_c	n_b	n_c	n_b	n_c	n_b	n_c	n_b	n_c
01	5.3	5.9	10.4	11.9	20.3	23.6	39.8	47.1	77.6	93.4
02	5.3	4.9	11.8	13.3	26.0	35.7	58.1	96.3	128.5	257.7
03	5.5	7.3	10.0	14.8	18.0	29.8	42.7	60.4	58.9	121.5
04	9.3	6.8	14.1	17.9	21.4	46.8	42.6	123.4	49.4	322.4
06	5.3	6.1	9.6	14.8	17.4	35.8	31.6	87.2	57.1	210.8

Top: k and g, parameters of the power function describing the relationship between number of breakage sites, $n_b(X)$ and particle size, X, using single-sized cubes (calibration experiments). m and h, parameters of the power function describing the relationship between critical particle number $n_c(X)$ and X using single-sized cubes. The values of these parameters have been assessed by curve-fitting of experimental data of $n_b(X)$ and $n_c(X)$, using a power function (*cf.* Fig. 3). Bottom: assessed, more accurate function values of $n_b(X)$ and $n_c(X)$ at various values of particle size X, according to the power-functions including their assessed values of their parameters (top). The values of the affinity factors $O_1(X,1)$ can be derived from the $n_c(X)$ values, using equation (12) (see text). These $n_b(X)$ and $O_1(X,1)$ values were used to predict the outcome of the experiments with simple particle mixtures according to the selection models. Subject no. 5 participated in another study, together with subjects no. 1-4 and 6 from the present study. Subjects no. 1, 2 and 6 are males and subject no. 3 and 4 are females.